

# Survey of Bovine Tuberculosis in Guduru Cattle Breeding and Research Center, Western Ethiopia

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## Abstract

In light of the direct correlation between *Mycobacterium bovis* (*M.bovis*) infection in cattle and the disease in human, control measures need to apply to reduce the prevalence of tuberculosis in developing countries. To this effect, generation of epidemiological data is of supreme importance. A cross-sectional study was conducted in line with this, at Guduru Cattle Breeding and Research Centre, Western Ethiopia, to estimate the prevalence of bovine tuberculosis (BTB) by using comparative intradermal tuberculin (CIDT) test. The prevalence was 1.8% (9/500) by using this test. There was no significant difference ( $\chi^2=4.75$ ;  $P=0.079$ ) in prevalence of BTB between Horro-Jersey cross breed and zebu (Horro) breed. The result of the present study has shown that bovine tuberculosis is less prevalent in cattle kept at Guduru Cattle Breeding and research Center. However, the prevalence of tuberculin result was low, enormous numbers of tubercle bacilli can be excreted by a cow with tuberculous mastitis. One cow can excrete enough viable bacilli to contaminate the milk of many cows, when their milk is mixed. Additionally it is much enough to contaminate an environment in which they belong (particularly pasture and water body), through faces and air droplet. In addition to this people of the area have the habit of consuming raw meat and milk and share the same microenvironment with their livestock. This further disseminates the causative agent, both through inhalation and ingestion resulting in high economic loss and public health effect. So further similar studies across the country to estimate the national prevalence of BTB is mandatory to design the control strategy.

**Keywords:** *Mycobacterium bovis*, tuberculin test, risk factors, Cattle, Ethiopia

## INTRODUCTION

Bovine tuberculosis (BTB) is described as a contagious, chronic and debilitating disease. The disease is caused by bacteria from the *mycobacterium tuberculosis* complex. The principal cause in cattle is *M.bovis* [1]. It is a fastidious organism that also causes tuberculosis in human and non-human primates, swine, goats, cats, dogs and many other domestic and wild animals [2]. The potential for transmission of zoonotic tuberculosis, that is, tuberculosis from animals to human occurs directly by aerosol and through the food chain by consumption of milk and rarely meat from tuberculous cattle. Milk products such as yoghurt, cream and cheese were also noted to have contained tubercle bacilli several days after being manufactured from unpasturized milk. As the main route of entry is oral route, tuberculosis of bovine origin in man is mainly extra pulmonary resulting in bone and joint tuberculosis as well as infection of the cervical and mesenteric lymph nodes [3, 4].

The tuberculin skin tests (TST) are currently the best available techniques for international field diagnosis of bovine TB in live animals [5, 6] and it is based on delayed hypersensitivity reactions [7]. The single intradermal comparative cervical tuberculin (SICCT) test involving the intradermal injection of bovine tuberculin (BT) and avian tuberculin (AT) at separate sites in the skin of the neck gives more specific results than the single intradermal tuberculin (SIT) test which uses only BT [8, 9]. TST can effectively detect early stages of *M. bovis* infection in cattle and allows for rapid removal of infected animals, limited transmission, and fast eradication of bovine TB [10].

Diagnosis of BTB is helpful to reduce the risk of Zoonosis, together with increasing public awareness and proper hygienic in food chain from animal source which may result in eradication [12]. Cattle and other bovine species are considered the primary and most well known reservoirs or maintenance host in countries where maintenance hosts are present endemically in the wild, infection from this population to the domestic cattle or other farm animals is difficult to avoid [13].

The tuberculin skin test may demand physical exertion in the field but, it is also simple and relatively inexpensive and offers reliable means of screening cattle populations in an entire region [8, 10]. Ancillary tests are being used and/or currently being validated to improve diagnosis and reduce the number of false positive results following TST [10, 11].

The performance of TST could be affected by environmental factors, host factors (status of immunity, genetics), and nature of the tuberculin used [8, 9]. A perfect cutoff point in a specific geographic area may not be so useful in another environment [5, 8]. Also, the ability of the test to predict positive disease status depends on its sensitivity and specificity and prevalence of the disease in tested population [5].

Although national data is not available on the prevalence of BTB in Ethiopia, it is assumed that the incidence of the disease is rising because of the present private-oriented economic policy of the government,

which thereby promotes the expansion of dairy industry. A few studies [24] have been conducted in central highlands of Ethiopia on the epidemiology of BTB, and the results of such studies have indicated that the disease is prevailing in these areas. Such studies were carried out most commonly using tuberculin skin testing, abattoir meat inspection and rarely on bacteriological techniques. Therefore, expansion of similar studies to the untouched regions of the country will be useful towards the establishment of the epidemiology of the disease at the national level. The current study was formulated to estimate the prevalence of BTB in a dairy herd kept at Guduru cattle breeding and research center, western Ethiopia,

#### **Objectives:**

- To estimate the prevalence of BTB in dairy herds kept at center.
- To assess the risk factors associated with the disease.

### **STUDY MATERIALS AND METHODS**

#### **Study area**

The tuberculin test was conducted in Guduru cattle breeding and research centre, which is located in Oromia National Regional state and about 275 km west of the capital city Addis Ababa along Gedo-Fincha sugar Factory high way. It is situated at an altitude of 2296 meters above the sea level, 09°9'N latitude and 37°26'E longitude. The area receives a unimodal type of rainfall.

Guduru ranch is suitable for livestock production. The predominant type of cattle is the Horro type and Horro-Jersey cross. Cattle production plays an important role in the centre for revenue generation and for distribution of improved heifers for the farmers. Currently the center was under the management of Wollega University for research as well as income generation to the University. There are about 1328 cattle (1178 pure Horro breed and 150 Horro-Jersey cross breed) in the center, which completely dependent on open grazing of the natural grass extensively that cover about 765 hectare of land. Except for the calves, the housing system is traditional on open air with fence only.

#### **Study design, Sample size and Sampling method**

The study design was cross-sectional by using skin test to determine the prevalence of BTB in the study area.

Taking in to an account the potential of the resource used for the test, 500 heads of cattle were determined to be included. Due to their relatively small proportion, all cross breed and male animals were included purposefully. The rest local female animals were selected by simple random method.

Sample size calculated based on 50 % prevalence assumption, 95% CI and  $p < 0.05$  [16]. Although the sample size calculated was 384, about 500 heads of cattle was tested due to the availability of resource.

$$n = \frac{Z^2 \cdot p_{\text{expe}} \cdot (1 - p_{\text{expe}})}{d^2}$$

Where n= required sample size

$P_{\text{expe}}$  =expected prevalence

d=Desired absolute precision (5 %)

Z= Normal distribution constant

#### **Study methodology**

##### **The comparative intra dermal tuberculin test**

Two sites on the right side of the mid-neck skin of the animals, 12cm apart, were shaved and skin thickness was measured using a caliper before injection of the purified protein derivative (PPD) and recorded as  $A_1$  for avian PPD site and  $B_2$  for bovine. One site was injected with an aliquot of 0.1ml of 20,000 IU/ml bovine PPD (*M. bovis*, AN5 strain) in to dermis toward the shoulder of an animal and other site was similarly injected with 0.1ml of 25,000 IU/ml of avian (D4 ER strain) PPD toward the head. Then their correct administration was checked if papula (pea like swelling) was formed and detected by palpation in the site of allergen inoculation. When the tuberculin was not administered intra dermally, the administration was repeated in the site in the prescribed dosage. After 72 hours, the skin at the injection site were again measured and recorded as  $A_2$  for avian PPD and  $B_2$  for bovine PPD. The same person should measure the skin fold thickness before and after tuberculin injection. The results were interpreted according to the recommendation of the office of International des epizooties [17].

##### **Interpretation**

A reaction was considered to be positive if the difference in skin fold thickness at the bovine site of injection was 4 mm or more higher than the reaction shown at the site of the avian injection.

When the difference in the skin fold thickness at the bovine site of inoculation was greater than 2 mm but lower than 4 mm, it was considered as doubtful, but if lower than or equal to 2 mm it was taken as negative [18].

If the increase was observed at both sites of the injection the difference was considered between the two sites of reactions. The increase in skin thickness at the injection site of avian  $\Delta$ PPD is  $\Delta$ PPD-A and is  $\Delta$ PPD-B for the reaction at site of bovine PPD. The manipulation of the change was done as this manner, subtracting  $\Delta$ PPD-A

from ΔPPD-B. When the change recorded as less than 2mm negative, between 2mm and 4mm suspected and greater than or equal to 4mm positives for bovine tuberculosis [17].

$\Delta\text{PPD-B} = [\text{Skin fold thickness after inoculation of PPD-B}] - [\text{Skin fold thickness before inoculation PPD-B}]$ .

$\Delta\text{PPD-A} = [\text{Skin fold thickness after inoculation of PPD-A}] - [\text{Skin fold thickness before inoculation PPD-A}]$ .

$\Delta (\text{PPD-B}) - \Delta (\text{PPD-A}) = > 4 \text{ mm}$ : Positive

$\Delta (\text{PPD-B}) - \Delta (\text{PPD-A}) = > 2 \text{ mm and } < 4 \text{ mm}$ : Doubtful

$\Delta (\text{PPD-B}) - \Delta (\text{PPD-A}) = < 2\text{mm}$ : Negative

### Data analysis

During the study, individual animal identification number, breeds, sex, age, were spreaded into MS Excel data sheets. Then, coded and were analyzed using SPSS version 20 statistical software. The prevalence rate was calculated by dividing the proportion of cattle found infected (either positive reactors or harbouring tuberculous lesions) by the total number of cattle tested multiplied by 100%. The risk factors associated with *M. bovis* infection were calculated by using Chi-square ( $\chi^2$ ) and logistic regression. Odds Ratio (OR) was assessed to investigate the strength of association. A statistically significant association between variables was said to exist if the calculated  $P < 0.05$ . For the analysis of the effect of different risk factors on bovine tuberculosis status of animals, doubtful reactors were not considered as positive [16].

## RESULTS

### Comparative intradermal tuberculin test

From the total of 500 cattle (358 local and 142 Horro-Jersey cross) tested for the presence of tuberculosis by the comparative intradermal tuberculin test 1.8% (9/500) were positive and 15(3%) determined as doubtful; the rest 476 (95.2%) were negative. On the other hand if we consider doubtful results as positive, the overall prevalence would be 4.8 % (24/500). Out of the nine positive cattle (considering doubtful as negative) to bovine tuberculosis, 8 were female and one was male animal. Six (1.7%) were of Horro breed and 3 (2.1%) were of the Horro-Jersey cross breed (Table 1). Twenty-five (5%) of the animals in the herd were reacted to both avian and bovine tuberculin.

## DISCUSSION

In the current study, the comparative intradermal tuberculin test (CIDT) showed the prevalence of BTB in Gudurru cattle breeding and research center was 1.8%. This is moderately lower than the previous studies conducted by [19] (3.4%) in West Wollega and Redi, 2003 (3.5%) at Assella in similar extensive management system. But there is a great difference when compared with tests conducted on intensive management [21, 22]. This variation might be due to the difference in the study animals used, study design and type of farming system. The disease is said to be more prevalent in dairy cattle kept under intensive management than others due to closer confinement, longer life spans and greater productivity stress [13, 23].

The present study site was established on the area which was previously used by near by farmers as a communal grazing land. There is an opportunity of contamination of the grass by parasitic egg and larvae from animal faces. Additionally there is swampy area in the middle of the ranch and the water used for the animals is stagnant damped for this purpose. According to [24], infection with different gastrointestinal parasites namely, *fasciola*, *paraphistomium*, *schistosoma* and *strongyle* has an effect on the immune response to tuberculin test. This is also supported by previous studies as *fasciola* and *strongyle* possess an external plasma membrane and particularly in their migratory larval stage, they are in close contact with the immune component such as antibody and the complement which act directly against them. Detailed study on the infection with *fasciola* showed that, antibody mediated cytotoxicity response are capable of killing the larval stage and young flukes as they migrate across the body cavity and through liver [25]. Similarly, nematodes such as *strongyle* release a wide variety of excretory or secretory antigens that are powerfully immunogenic. Therefore, infection with *fasciola* or *strongyle* has induced strong Th2 cell response there by inhibiting Th1 cell response to tuberculin injection [26, 27].

The comparison made between crosses and local cattle supports the idea that, cross cattle are found to react more likely to tuberculin than local (1.7% in local and 2.1% in cross breed ). But, the difference is not as high as the one, seen between local and local-Holstein Friesian cross. The strength of association (OR) was calculated considering sex as a risky factor, where crosses were 5 times more likely to develop tuberculosis than local breeds. The possible explanation for this might be stress of high production.

However, there is biological association as observed from the Odd's ratio value, the test revealed no statistical significant difference. The possible reason might be due to in proportionality in the number of the animal compared in specific variable.

## CONCLUSIONS AND RECOMMENDATIONS

The result of the present study has shown that bovine tuberculosis is less prevalent in cattle kept at Guduru Cattle Breeding and research Center. However, the prevalence of tuberculin result was low, enormous numbers of tubercle bacilli can be excreted by a cow with tuberculous mastitis. One cow can excrete enough viable bacilli to contaminate the milk of many cows, when their milk is mixed. Additionally it is much enough to contaminate an environment in which they belong (particularly pasture and water body), through faces and air droplet. In addition to this people of the area have the habit of consuming raw meat and milk and share the same microenvironment with their livestock. This further disseminates the causative agent, both through inhalation and ingestion resulting in high economic loss and public health effect. So that,

Similar studies across the country, further characterization of the causative agent and Public education to increase the awareness of the community about the potential risk of consumption of raw animal products is necessary.

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## ANNEX

**Table 1:** Host risk factors associated with *M. bovis* infection

Variables		No- of animals			$\chi^2$	P-value
		Positive	Doubtful	Negative		
Age	≤5 years	6	13	253	7.719	0.076
	5 -10 years	3	1	181		
	>10 years	-	1	42		
BCS	Lean	3	2	138	2.543	0.643
	Medium	5	11	251		
	Fat	1	2	87		
Breed	Local	6	7	345	4.746	0.079
	Cross	3	8	131		
Sex	Female	8	11	335	1.220	0.594
	Male	1	4	141		
<b>Total</b>		<b>9</b>	<b>15</b>	<b>476</b>		



**Fig 1.** Tuberculin reaction

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